



Figure 2 a

PROCESS DESCRIPTION

UNIT OPERATION

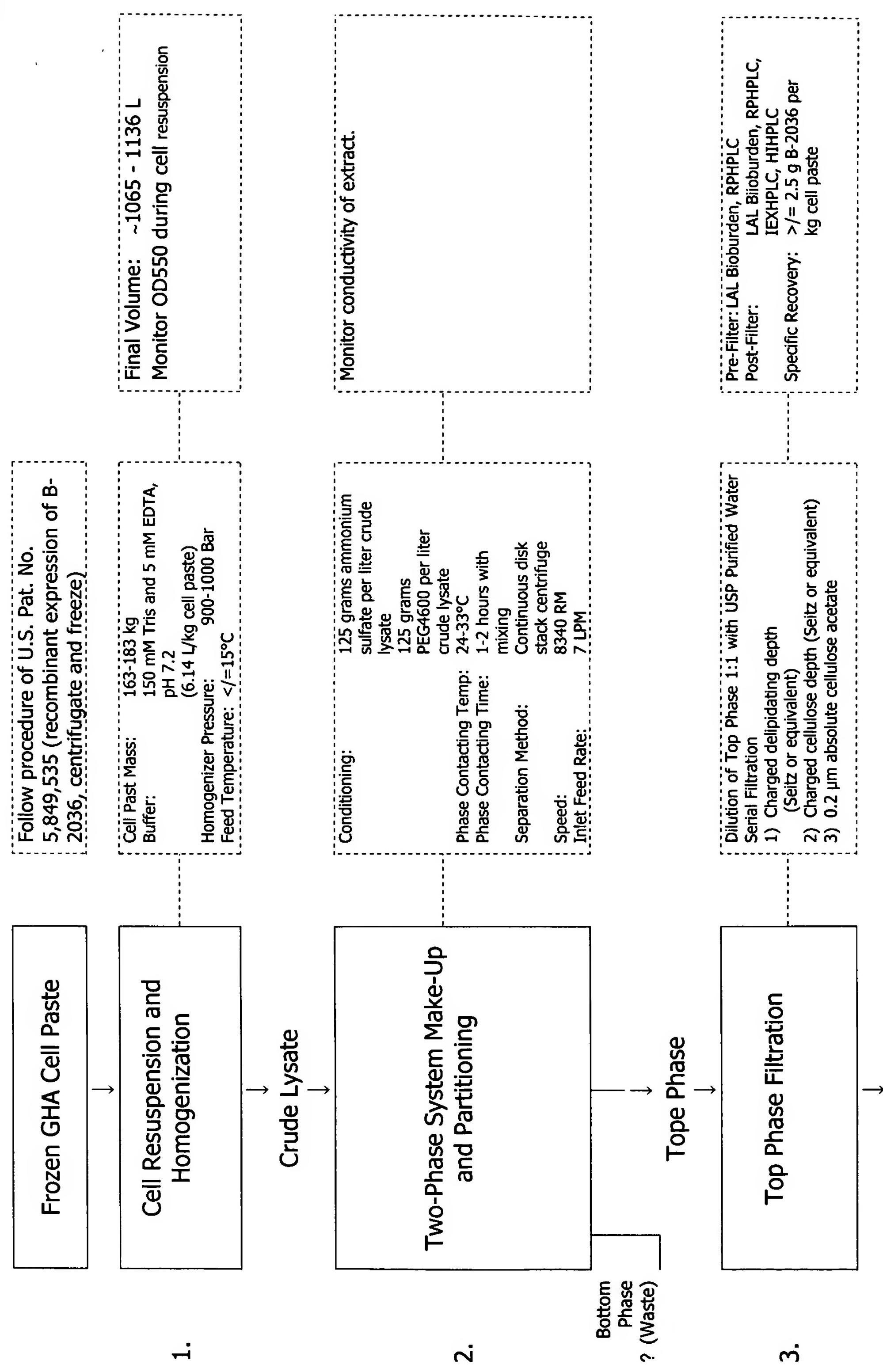


Figure 2 b

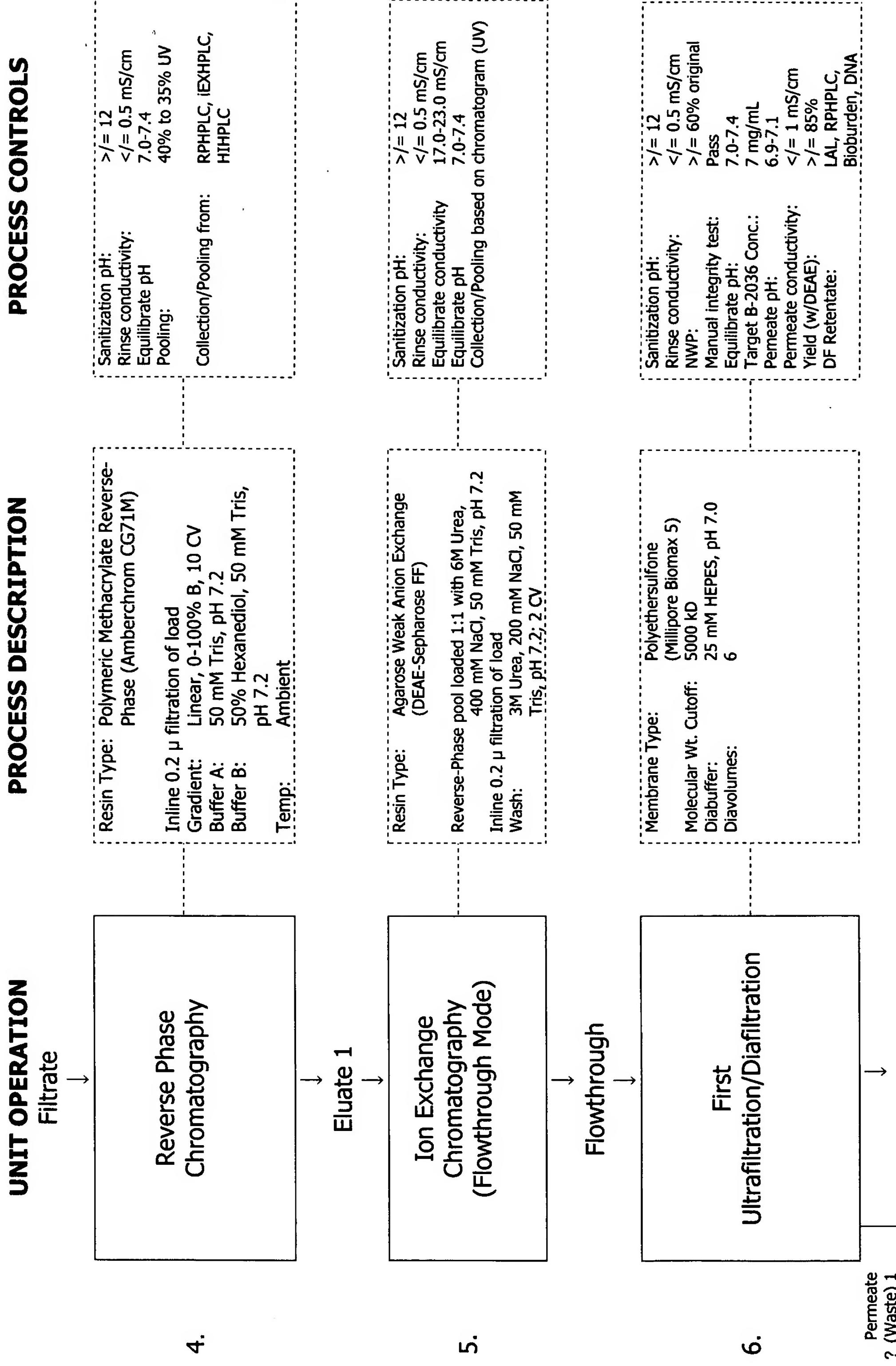


Figure 2 C

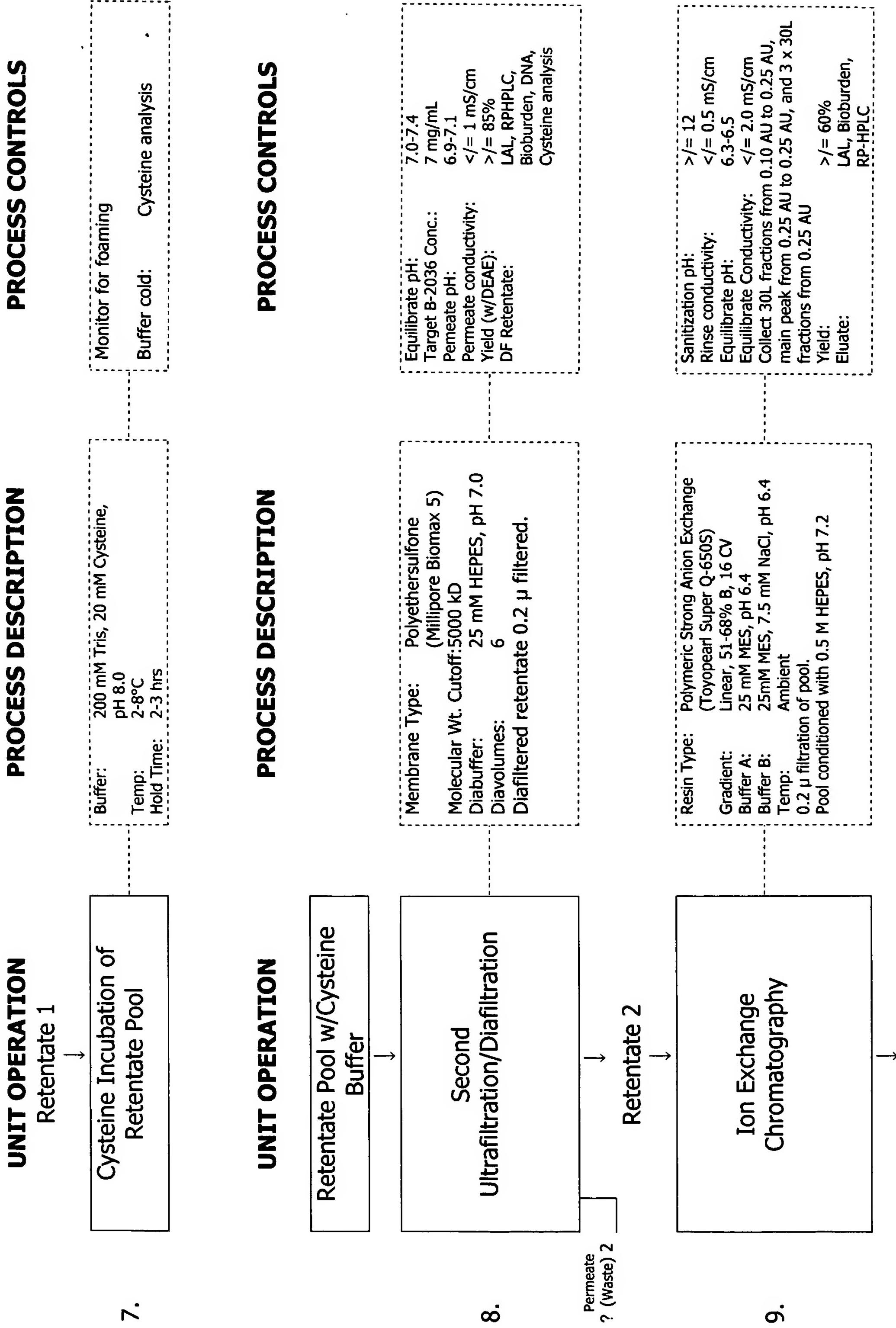


Figure 2 d

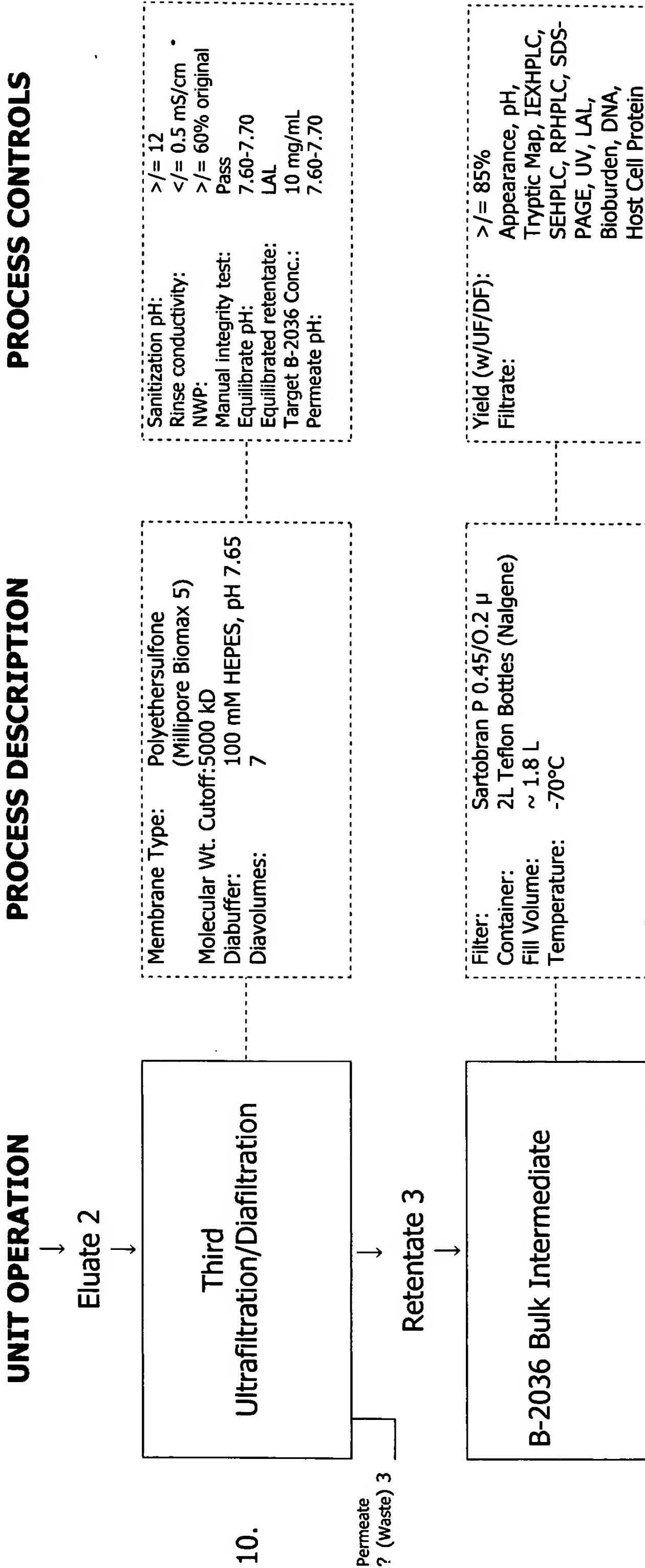


Figure 3 a

PROCESS DESCRIPTION

UNIT OPERATION

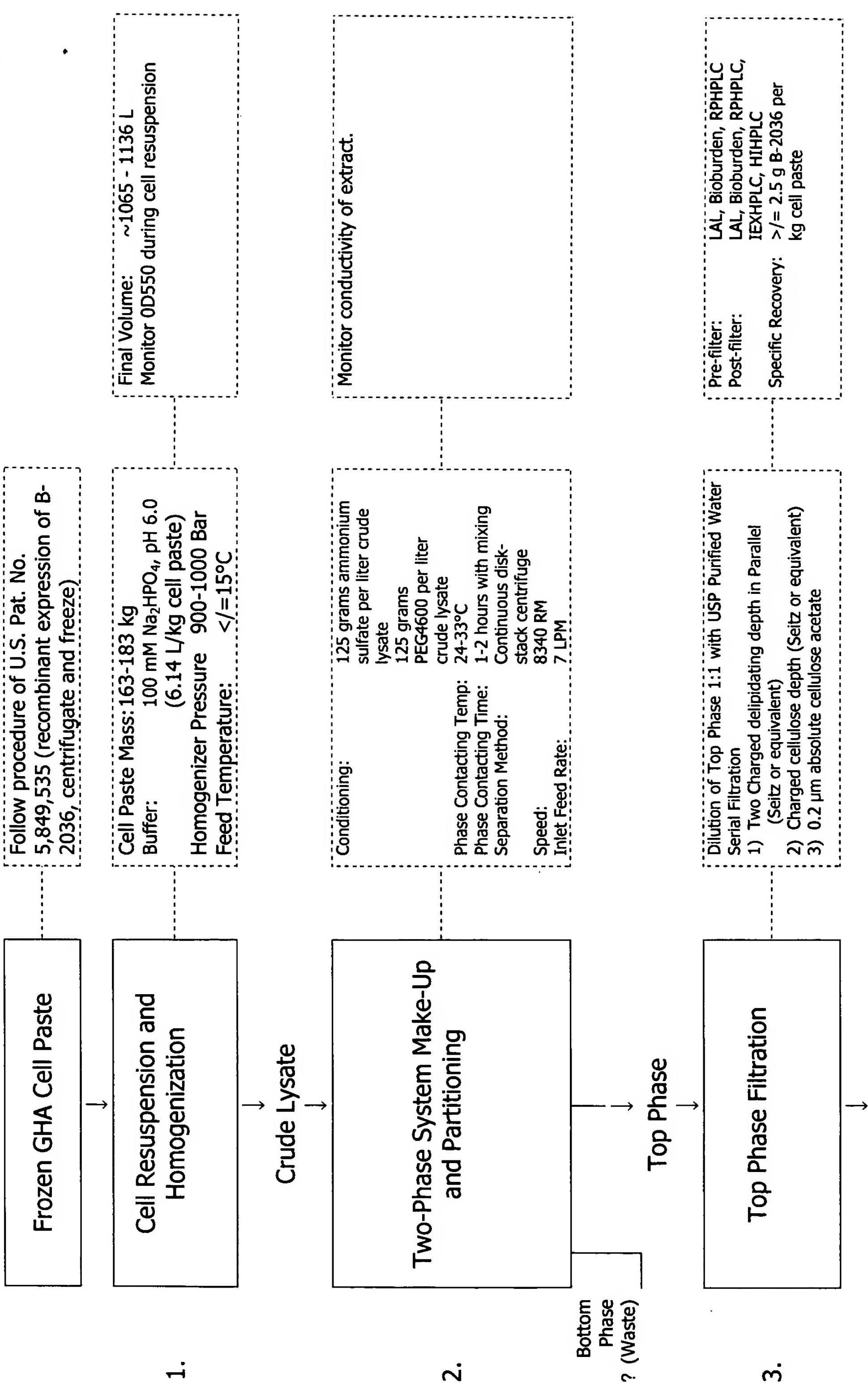


Figure 3 b

PROCESS DESCRIPTION

PROCESS CONTROLS

UNIT OPERATION

Filtrate

Reverse-Phase Chromatography

4.

Resin Type:	Polymeric Methacrylate Reverse Phase (Amberchrom CG71M)
Inline 0.2 μ filtration of load	
Gradient:	Linear, 0-100% B, 10 CV
Buffer A:	50 mM Tris, pH 7.2
Buffer B:	50% Hexanediol, 50 mM Tris, pH 7.2
Temp:	Ambient

↓
Eluate 1

First Ion Exchange Chromatography (Flowthrough Mode)

5.

Resin Type:	Agarose Weak Anion Exchange (DEAE-Sepharose FF)
Reverse-Phase pool loaded 1:1 with 6M Urea, 400 mM NaCl, 50 mM Tris, pH 7.2	
Inline 0.2 μ filtration of load	
Wash:	3M Urea, 200 mM NaCl, 50 mM Tris, pH 7.2; 2 CV

↓
Flowthrough

First Ultrafiltration/Diafiltration

6.

Membrane Type:	Polyethersulfone (Millipore Biomax 5)
Molecular Wt. Cutoff:	5000 kD
Diabuffer:	25 mM HEPES, pH 7.0
Diavolumes:	6
Diafiltered retentate	0.2 μ filtered.

↓
Retentate 1

Permeate
? (Waste)

Sanitization pH:	>/= 12
Rinse conductivity:	</= 0.5 mS/cm
Equilibrate pH:	7.0-7.4
Pooling:	40% - 35% UV
Pool:	RPHPLC, IEXHPLC, HIHPLC

Sanitization pH:	>/= 12
Rinse conductivity:	</= 0.5 mS/cm
NWP:	>/= 60% original
Manual integrity test:	Pass
Equilibrate pH:	7.0-7.4
Target B-2036 Conc.:	7 mg/mL
Permeate pH:	6.9-7.1
Permeate conductivity:	</= 1 mS/cm
Yield (w/DEAE):	>/= 85%
DF Retentate:	LAL, RPHPLC, Bioburden, DNA

Figure 3 C

PROCESS CONTROLS

PROCESS DESCRIPTION

UNIT OPERATION

**Ultrafiltration/Diafiltration
Retentate 1**



**Second Ion Exchange
Chromatography**

7.

Resin Type: Polymeric Strong Anion Exchange (Toyopearl Super Q-650S)
Gradient: Linear, 51-68% B, 16 CV
Buffer A: 25 mM MES, pH 6.4
Buffer B: 25mM MES, 7.5 mM NaCl, pH 6.4
Temp: Ambient
0.2 μ filtration of pool.
Pool conditioned with 0.5 M HEPES, pH 7.2

Eluate 2

8.

**Second
Ultrafiltration/Diafiltration**

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
Equilibrate pH: 6.3-6.5
Equilibrate Conductivity: </= 2.0 mS/cm
Pooling: 0.25 to 0.25 AU
Eluate: LAL, Bioburden, RP-HPLC, IEX-HPLC, HI-HPLC, UV Scan

Membrane Type: Polyethersulfone (Millipore Biomax 5)
Molecular Wt. Cutoff: 5000 kD
Diabuffer: 100 mM HEPES, pH 7.65
Diavolumes: 7

Retentate 2

**Permeate
? (Waste)**

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
NWP: >/= 60% original
Manual integrity test:
Pass
Equilibrate pH: 7.60-7.70
Equilibrated retentate:
Target B-2036 Conc.: 10 mg/mL
Permeate pH: 7.60-7.70

Yield (w/UFD/DF): > = 85%
Filtrate:
Appearance, pH,
Tryptic Map, IEXHPLC,
SEHPLC, RPHPLC, SDS-
PAGE, UV, LAL,
Bioburden, DNA,
Host Cell Protein

B-2036 Bulk Intermediate